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Research article

Proteomics analysis of plasm exosomes in early pregnancy among normal pregnant women and those with antiphospholipid syndrome

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ABSTRACT

Introduction: Antiphospholipid syndrome (APS) is an autoimmune disorder associated with thrombosis and adverse obstetric outcomes. Early diagnosis and intervention can improve pregnancy outcomes to some extent, but current results are unsatisfactory. Exosomes, containing biomacromolecules relevant to reproduction, play essential roles in pregnancy. However, research progress on their involvement in APS remains limited.

Objectives: This study aims to investigate protein profile changes in plasma exosomes and identify potential biomarkers for obstetric APS.

Methods: We employed tandem mass tag (TMT) markers to analyze exosome protein profiles from 6 healthy early pregnant women and 6 early-stage APS patients. Quantitative proteomics analysis was conducted using the Maxquant search engine.

Results: Differential expression analysis identified 51 upregulated and 22 downregulated proteins in plasma exosomes from early pregnant women with APS, such as serpin peptidase inhibitor C1/A1/A7, apolipoprotein 1/2, orosomucoid 1/2 and apolipoprotein H. Kyoto Encyclopedia of Genes and Genomes analysis shows that differentially expressed proteins are enriched in the PPAR signaling pathway and staphylococcus aureus infection pathway. Enrichment analysis indicated associations with glycerolipid biosynthesis, vitamin transport, and negative regulation of very-low-density lipoprotein particle remodeling.

Conclusion: Our study highlights alterations in the protein profiles of plasma exosomes in APS pregnant patients and proposes potential biomarkers, offering insights for early diagnosis and treatment and improving reproductive outcomes.

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1. Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by arterial or venous thrombosis, decreased platelets, and pathological pregnancies. Current treatment effectiveness remains around 70 % [1–4] [1–4] [1–4]. Early diagnosis of APS poses challenges, adversely affecting obstetric outcomes for patients [2]. Hence, this study aims to identify biomarkers for early diagnosis of APS during pregnancy through proteomics analysis of plasma exosomes. This has implications for exploring precision clinical treatments and also contributes to exploring new directions in APS therapy.

This study specifically focuses on obstetric APS (OAPS), characterized by recurrent fetal loss, early fetal death, or preterm delivery before 34 weeks due to preeclampsia, eclampsia, or placental insufficiency [4,5]. Early identification of such cases is crucial for successful pregnancy outcomes [2]. Diagnosis of APS currently relies on the presence of vascular thrombosis, pregnancy complications, and detection of antiphospholipid antibodies (aPLs) [6,7]. However, the lack of standardization in aPLs testing methods across different medical institutions and the heterogeneity of aPLs themselves contribute to poor reproducibility and difficulty in laboratory test standardization [8]. Standard treatments involving prednisolone, low-dose aspirin (LDA), and heparin significantly improve outcomes for APS patients [8–10] [8–10] [8–10]. However, the success rate of pregnancies in treated APS patients ranges from 70 % to 85 %, with a high prevalence of preterm birth, preeclampsia, eclampsia, and peri-thrombotic events [2]. The efficacy of these measures in significantly improving the prognosis of APS patients is often limited. Therefore, there is an urgent need to explore additional biomarkers, especially those reflecting pregnancy morbidity and prothrombotic states.

Previous studies suggest that circulating exosomes derived from peripheral blood in APS patients are detrimental to vascular development and can lead to pregnancy complications [11]. Additionally, extracellular vesicles (EVs) have been implicated in coagulation and inflammation [12]. Recent research indicates elevated levels of circulating EVs in APS patients, possibly related to systemic vascular activation [12]. Although direct evidence linking increased EVs levels to thrombosis and obstetric events in APS is lacking, EVs may participate in the pathogenesis of APS by promoting vascular activation and thrombosis [13]. Exosomes, a subtype of EVs ranging from 30 to 200 nm in diameter, have been implicated in various reproductive processes, suggesting significant potential in reproductive disease diagnosis and treatment [14,15]. In addition, exosomes proteins serve as important biomarkers for reproductive system diseases such as endometrial cancer and ovarian cancer [16]. Utilizing exosomes proteins as biomarkers could greatly enhance our understanding of reproductive dysfunction and related diseases.

In this study, we conducted proteomics analysis of exosomes derived from APS and non-APS pregnant women. The results of this study can provide reference for early diagnostic biomarkers research of OAPS.

2. Materials and methods

2.1. Sample collection

In this study, we collected peripheral blood plasma from normal pregnant women and APS patients in early pregnancy (start of the last menstrual period through the end of 12 weeks of gestation) to extract exosomes for the identification of their protein characteristics. All subjects had their peripheral blood collected in a fasting state in the morning. Subsequently, the blood samples were quickly transferred into heparin-containing anticoagulation tubes and mixed by vortexing or inverting 5 to 10 times.

Table 1
Clinical characteristics of the APS group and NC group. (If the content of antibody was below 2 in the laboratory tests, then the result was negative. For analyzing more conveniently, these negative results were regarded as 2.)In the APS group, all patients received the standard treatment regimen, which includes: (1) Prednisone (produced by Shanghai Xinyi Pharmaceutical Co., Ltd.) taken orally, starting from the pre-pregnancy period, once daily. The initial dose is 5 mg, with a general medication dose ranging from 5 to 15 mg, continuing until the end of pregnancy; (2) Aspirin (produced by Shanghai Xinyi Pharmaceutical Co., Ltd.) taken orally, starting from the confirmation of pregnancy, once daily. The initial dose is 25 mg, with a general medication dose ranging from 25 to 75 mg, continuing until three days before delivery; (3) Low molecular weight heparin (produced by Hangzhou Jiuyuan Gene Engineering Co., Ltd.) administered via intramuscular injection, starting from the confirmation of pregnancy, three times daily. The initial dose is 5000 U/d, with a general medication dose of 5000U/d, continuing until the 35th week of pregnancy. Regarding outcomes, all patients in the control group had full-term vaginal deliveries, while in the APS group, there was one full-term delivery, one delivery at 32 weeks, one case of premature rupture of membranes, one early miscarriage, and two cases where outcome data were missing.

	NC group (Mean ± SEM)	APS group (Mean ± SEM)	*P*
Count	6	6	
Age (year)	28.33 ± 1.764	27.83 ± 0.703	>0.05
Gestational age (day)	42.33 ± 2.951	41.00 ± 2.309	>0.05
Times of abortion	0	2.167 ± 0.167	<0.05
Antiphospholipid antibody			
Anti-apoh IgM antibody (AU/ml)	2	52.92 ± 4.565	<0.05
Anti-apoh IgG antibody (AU/ml)	2	2	
Anticardiolipin antibody IgM antibody (MPL/ml)	2	2	
Anticardiolipin antibody IgG antibody (GPL/ml)	2	2	
Anti-lupus anticoagulant(S)	negative	positive	

2.1.1. Participants

From July 2018 to December 2018, our study enrolled a total of 12 pregnant women in the early stages of pregnancy: 6 healthy individuals (NC group) and 6 with APS (APS group), all receiving care at the First Maternal and Infant Health Hospital Affiliated with Tongji University (Table 1).

2.1.2. Inclusion and exclusion criteria for the APS group

For the diagnosis of patients with OAPS, we followed the criteria set forth in the "American Society for Reproductive Medicine Technical Committee and the Society for Reproductive Medicine Consensus," where clinical criteria are consistent with a history of pathological pregnancy and are confirmed through laboratory tests as positive for β2-glycoprotein 1 (β2-GP1) antibodies. Pathological pregnancy includes: (1) one or more unexplained cases where a morphologically normal fetus is confirmed by ultrasound or direct fetal examination to be dead in utero after more than 10 weeks of pregnancy; (2) one or more cases where a morphologically normal fetus is delivered prematurely before 34 weeks of pregnancy due to severe preeclampsia or severe placental dysfunction; (3) two or more consecutive unexplained miscarriages within 10 weeks of pregnancy. Meeting any one of these criteria suffices. Laboratory criteria: The presence of medium or high titers of IgG or IgM *anti*-β2-GP1 antibodies in the blood on two or more occasions at least 12 weeks apart [17,18].

Pregnant women diagnosed with OAPS were included in the APS group. The participants ranged from 20 to 40 years old, were between 6 and 8 weeks gestational age.

Those with comorbidities such as uterine fibroids, ovarian cysts, endocrine disorders, and infections were excluded from the study. Patients with other autoimmune diseases, thrombotic disorders, and other serious comorbidities are also excluded.

2.1.3. Inclusion and exclusion criteria for the normal control (NC) group

Those within the age range of 20–40 years, who have confirmed intrauterine pregnancy through B-ultrasound and are in their gestational period between 6 and 8 weeks without any history of spontaneous abortion, preeclampsia, eclampsia or preterm birth were included in the NC group.

Those with uterine fibroids, ovarian cysts, endocrine abnormalities, infections, and other acute or chronic illnesses, as well as those who tested positive for antiphospholipid antibodies were excluded from the study.

2.1.4. Extraction of exosomes

Immediately proceed with the extraction of exosomes following sample collection. Initially, the plasma samples were briefly centrifuged at 3000 g for 5 min at 4 °C to remove any large cell fragments or debris. The supernatant was then subjected to further centrifugation at 10000*g* for 60 min at 4 °C (Avanti J-26S XP) in order to extract the small vesicles. To remove small cellular debris, the supernatant was collected again and mixed with phosphate-buffered saline (PBS, Biological Industries, Israel). The mixture was then centrifuged at 10000 g for 120 min at 4 °C using an OptimaXE-90 ultracentrifuge. Finally, the supernatant was discarded and the remaining pellet was resuspended in PBS before being stored at −80 °C.

2.2. Protein extraction and quantification

Samples were removed from −80 °C, and each group of samples was respectively added to four volumes of lysis buffer (8 M urea, 100 mM Tris-HCl, pH 8.0, 1 % protease inhibitor) and lysed using an ultrasonic cell disruptor JY92-IIN (Ningbo Xinzhi) at 20 % power, with 3s sonication in an ice-water bath followed by a 2s pause, for a total sonication time of 3 min. Centrifugation was performed at 4 °C, 20,000 g for 10 min, and the supernatant was collected and mixed with trichloroacetic acid to a final concentration of 20 %, then left to stand at 4 °C for 2 h. Afterward, centrifugation at 4 °C, 12,000 g for 3 min was done, the supernatant was discarded, and the precipitate was washed three times with pre-cooled acetone. Finally, the precipitate was redissolved in 8 M urea. Protein concentration was determined using a bicinchoninic acid (BCA) assay kit (Beyotime P0010) as follows:

Take 5 μL of the protein sample and determine the protein concentration using a BCA assay kit. The method is as follows: (1) Add standard samples at volumes of 0 μL, 5 μL, 10 μL, 15 μL, and 20 μL to the wells of an enzyme label plate, fill up to 20 μL with sample diluent, and run three replicates for each; (2) Add 5 μL of the protein sample to be tested to the wells of the enzyme label plate, fill up to 20 μL with sample diluent, and run three replicates for each; (3) Add 200 μL of BCA working solution to each well and incubate at 37 °C for 30 min; (4) Measure A570 with an enzyme label meter (the optimal absorption wavelength is 562 nm, but other wavelengths between 540 and 595 nm can also be applied); (5) Calculate the protein concentration of the samples based on the standard curve and the volume of sample used.

2.3. Trypsin digestion

The protein solution was reduced with 5 mM dithiothreitol at 56 °C for 30 min, followed by alkylation with 11 mM iodoacetamide in the dark at room temperature for 15 min prior to digestion. Once the urea concentration dropped below 2 M, the protein sample was diluted with 100 mM tetraethyl ammonium bromide (TEAB). Subsequently, trypsin was added at a ratio of 1:50 for the initial overnight digestion and a ratio of 1:100 for a second 4-h digestion.

2.4. Proteomics analysis

In this part, samples were processed for total-protein tandem mass tag (TMT) labeling by Jingjie PTM BioLab (Hangzhou, China).

2.4.1. TMT labeling

Peptides digested by trypsin were desalted using Strata X C18 (Phenomenex) and then lyophilized under vacuum. The peptides were dissolved in 0.5 M TEAB and labeled according to the instructions of the TMT reagent kit. The procedure is as follows: After thawing, the labeling reagent was dissolved in acetonitrile, mixed with the peptides, and then incubated at room temperature for 2 h. After labeling, the peptides were desalted and lyophilized under vacuum.

2.4.2. High performance liquid chromatography (HPLC)

Fractionation Peptides were fractionated using high pH reverse HPLC, with the chromatography column being Agilent 300Extend C18 (5 μm particle size, 4.6 mm inner diameter, 250 mm length). The operation is as follows: The gradient for peptide fractionation was from 8 % to 32 % acetonitrile, pH 9, over 60 min to separate 60 components, which were then combined into 9 fractions. These combined fractions were lyophilized under vacuum for further processing.

2.4.3. Liquid chromatography-mass spectrometry (LC-MS) analysis

The peptides were dissolved in liquid chromatography mobile phase A (0.1 % (v/v) formic acid in water) and separated using the EASY-nLC 1000 ultra high performance liquid system. Mobile phase A consisted of 0.1 % formic acid and 2 % acetonitrile in water; Mobile phase B consisted of 0.1 % formic acid and 90 % acetonitrile in water. The liquid chromatography gradient was set as follows: from 0 to 26 min, 9%–25 % B; from 26 to 34 min, 25%–36 % B; from 34 to 37 min, 36%–80 % B; from 37 to 40 min, 80 % B, with the flow rate maintained at 700 nL/min. After separation by the ultra high performance liquid system, the peptides were ionized in the NSI ion source and then analyzed by the Orbitrap FusionTM mass spectrometer. The ion source voltage was set at 2.0 kV, and both the precursor ions and their secondary fragments were detected and analyzed using the high-resolution Orbitrap. The scan range for the primary mass spectrometry was set to 350–1550 *m/z*, with a resolution of 60,000; the secondary mass spectrometry scan range started at 100 *m/z*, with an Orbitrap resolution of 15,000. The data acquisition mode used was a data-dependent scan (DDA) program, where after a primary scan, the top 20 precursor ions with the highest signal strength were selected to enter the HCD collision cell for fragmentation with 35 % fragmentation energy, followed by sequential secondary mass spectrometry analysis. To improve the effective utilization rate of mass spectrometry, the automatic gain control (AGC) was set to 5E4, the signal threshold was set to 5000 ions/s, the maximum injection time was set to 200 ms, and the dynamic exclusion time for tandem mass spectrometry scans was set to 30 s to avoid repeated scanning of parent ions.

2.4.4. Database search

The secondary mass spectrometry data was searched using Maxquant (v1.5.2.8). The search parameters were set as follows: the database was SwissProt Human (20130 sequences), with a decoy database added to calculate the false positive rate (FDR) caused by random matches, and a common contaminant database was included to eliminate the impact of contaminant proteins in the identification results; the enzymatic digestion was set to Trypsin/P; the number of missed cleavage sites was set to 2; the minimum length of peptides was set to 7 amino acid residues; the maximum number of modifications per peptide was set to 5; the mass tolerance for the first and main search of precursor ions was set to 20 ppm and 5 ppm, respectively, and the mass tolerance for secondary fragment ions was set to 0.02 Da. Cysteine alkylation was set as a fixed modification, with variable modifications being methionine oxidation and protein N-terminal acetylation. The quantification method was set to TMT-6plex, with both protein identification and PSM identification FDRs set at 1 % [19].

2.4.5. Mass spectrometry quality control

First, we examined the mass deviation (mass error) of all identified peptides. The mass errors were centered around zero and concentrated within a range of less than 10 ppm, indicating that the mass accuracy meets the requirements. Secondly, the majority of peptide lengths were distributed between 8 and 20 amino acid residues, consistent with the pattern of peptides digested by trypsin, indicating that the sample preparation met the standard.

2.5. Bioinformatics analysis methods and statistical analysis

2.5.1. Protein annotation methods

Gene Ontology (GO) annotations at the proteomics level come from the UniProt-GOA database (www.ebi.ac.uk/GOA/). Initially, protein IDs are converted to UniProt IDs, which are then used to match GO IDs, and based on these GO IDs, relevant information is retrieved from the UniProt-GOA database. If the UniProt-GOA database does not contain information on the queried protein, we use a protein sequence-based algorithm software, InterProScan, to predict the GO functions of the protein. The proteins are then classified according to cellular components, molecular functions, or biological processes.

For project data, the software InterProScan and the corresponding InterPro domain database (http://www.ebi.ac.uk/interpro/) are used to annotate the protein domains of the identified proteins.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database is used for annotating protein pathways: firstly, the submitted proteins are annotated using the KEGG online service tool KEGG Automatic Annotation Server (KAAS), then the annotated

proteins are matched to corresponding pathways in the database through KEGG mapper [20].

The software wolfpsort, which predicts subcellular localization, is used to annotate the subcellular localization of the submitted proteins.

2.5.2. Protein function enrichment

GO annotations of proteins are divided into three main categories: biological processes, cellular components, and molecular

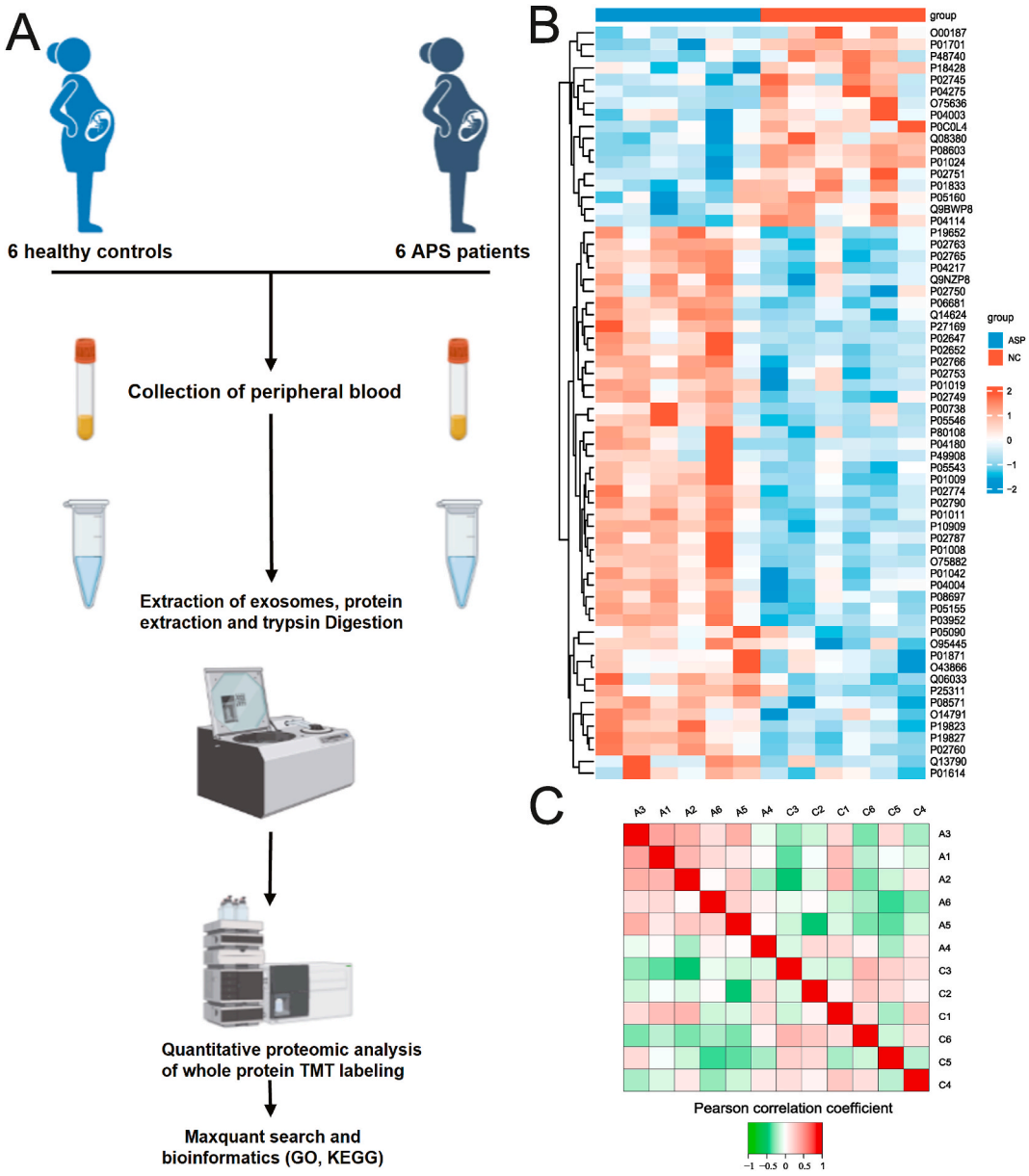

Fig. 1. The basic procedure of the study and the identification of DEPs.(A) Overview of the experimental design and proteomics analytical processes. Plasm exosomes were isolated from healthy pregnant women or APS patients, followed by a total-protein TMT labeling and quantitative proteomics analysis to determine the abundance of exosome proteins. The data were subjected to analysis using a series of bioinformatics tools. (B) Heatmap showing 66 effective DEPs in the APS group and NC group, for that ones with censored data were excluded among 73 DEPs. Each protein is represented by a row, and each sample by a column. Varied hues on the heatmap represented varied protein expression levels, with red denoting proteins with high expression and blue denoting proteins with low expression. (C) Heatmap according to Pearson's correlation coefficients among 12 samples.. (Pearson's correlation coefficients, which is a measure of the degree of linear correlation between two sets of data, when it is close to −1, the two sets of data present a negative correlation. On the contrary, when the coefficient tends to be 1, the two sets of data present a positive correlation, and the closer it is to 0, the it is more possible that the two sets of data is no correlation.). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

functions. Fisher's exact test is used to test the differential expression proteins against the background of identified proteins. A GO enrichment test *P*-value of less than 0.05 is considered significant.

The KEGG database is used for pathway enrichment analysis. Fisher's exact test is used to test the differential expression proteins against the background of identified proteins. A pathway enrichment test *P*-value of less than 0.05 is considered significant. Finally, these pathways are categorized according to the KEGG website's pathway hierarchy classification method.

The InterPro database (which provides resources for the analysis of protein families, domains, and functional sites) is used to analyze the enrichment of functional domains in differentially expressed proteins. Fisher's exact test is used to test the differential expression proteins against the background of identified proteins. An enrichment test *P*-value of less than 0.05 for domain units is considered significant.

2.5.3. Clustering analysis based on protein function enrichment

First, we collect the functional classification information and corresponding enrichment *P*-value of all protein groups, then select the functional classifications that are significantly enriched (*P*-value <0.05) in at least one protein group. The selected *P*-value data matrix is first transformed using a logarithm of -log10, and then the transformed data matrix is subjected to a Z-transform for each functional classification. Finally, the dataset obtained after the Z-transform is subjected to hierarchical clustering (Euclidean distance, average linkage clustering) for unilateral cluster analysis. The clustering relationships are visualized using the heatmap.2 function from the R package gplots.

2.5.4. Statistical analysis and protein-protein interaction (PPI) network

Furthermore, we constructed a PPI network of differentially expressed proteins (DEPs) by utilizing the search tool for the retrieval of interacting genes/proteins (STRING) online database (v11.5) [21].

GraphPad Prism 6.0 was utilized for statistical analysis. The clinical information of participants is presented as the mean ± standard error of the mean (SEM). T-test was performed to estimate significant differences between groups, and differences were considered statistically significant when *P*-value was <0.05.

2.6. Ethics statement

The studies involving participants were conducted in accordance with the ethical policies and procedures approved by the Ethics Committee of Shanghai First Maternal and Infant Hospital affiliated with Tongji University (KS22212). Written informed consent was obtained from all participants.

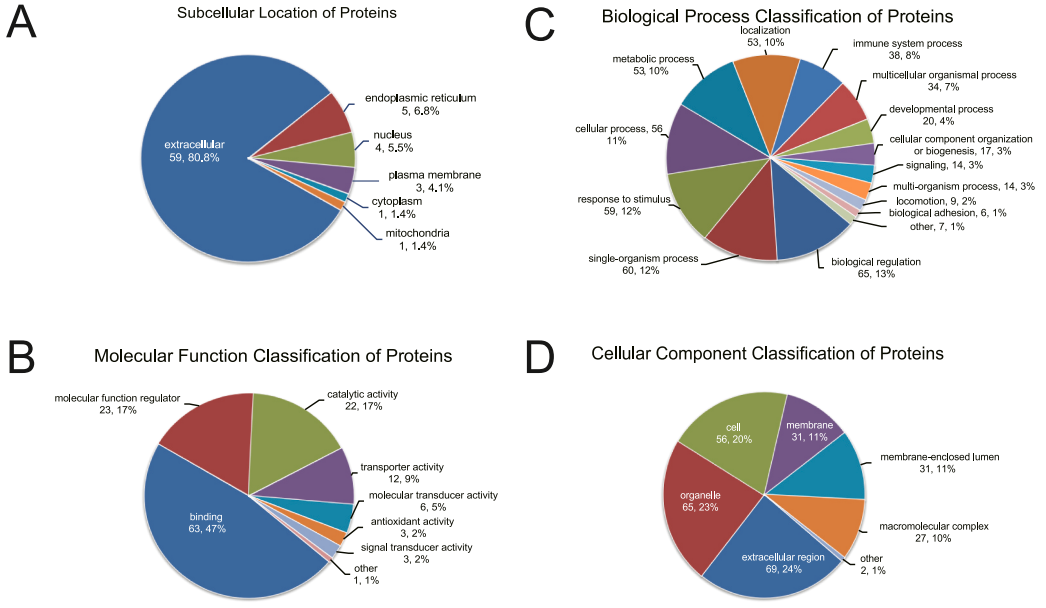

Fig. 2. Functional classification of proteins according to GO annotation. (A) Pie-chart of the sub-cellular location of proteins. (B) Pie-chart of biology process classification of proteins. (C) Pie-chart of molecular function classification of proteins. (D) Pie-chart of cellular component classification of proteins.

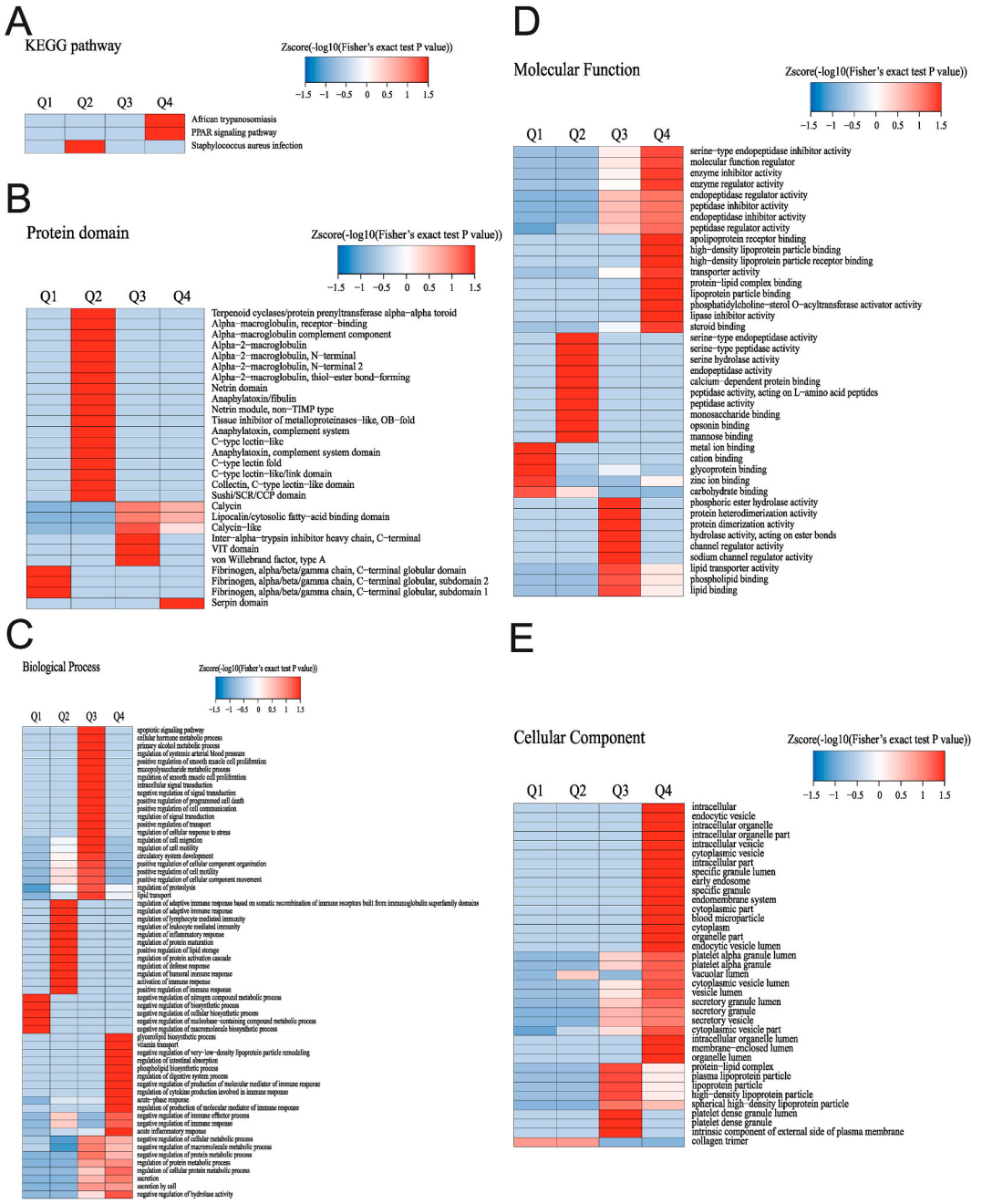

Fig. 3. Heatmap of clustering analysis of DEPs in APS. Utilizing the p-values derived from enrichment analysis via Fisher's exact test, we employed hierarchical clustering to aggregate associated functions across distinct groups, visually represented in a heatmap format. The horizontal axis of the heatmap illustrates the results of the enrichment tests for the various groups, whereas the vertical axis details the descriptions of functions enriched by differential expression, encompassing categories like GO, KEGG pathways, and protein domains. The intensity of the color blocks, aligning with the differential expression proteins and their corresponding functional descriptions across groups, signifies the level of enrichment; with red indicating higher enrichment and blue denoting lower enrichment. (A) Heatmap of KEGG pathway enrichment analysis among 4 clusters. (B) Heatmap of protein domain enrichment analysis among 4 clusters. (C–E) Heatmap of GO enrichment analysis among 4 clusters comprise biological process (C), molecular function (D), and cellular component (E). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Result

3.1. Clinical characteristics of the APS group and NC group

The diagnostic criteria of APS were based on the 2006 international consensus statement [2]. Clinical characteristics of the APS group and NC group are presented in Table 1. There was no significant difference ($P > 0.05$) in age (25–35 years old) and gestational age (6–8 weeks) between participants in the NC and APS groups.

3.2. Proteomics profiling and identification of DEPs in plasm exosomes

Fig. 1, A depicts the experimental design and proteomics analytical processes employed in this study. In the course of quantitative proteomics analysis, 293 proteins were identified, out of which 261 had quantifiable information (Supplement Table 1). In this study, a change threshold of 1.2-fold was applied and only proteins with a *t*-test *P*-value<0.05 were considered as DEPs. As such, we identified 51 significantly up-regulated and 22 down-regulated DEPs in the APS group compared to the NC group (Fig. 1B–Supplement Table 2). To ensure data quality, we conducted sample repeatability tests and calculated Pearson's correlation coefficients for all samples, which were then used to generate a heatmap (Fig. 1C–Supplement Table 3). Based on the primary analysis, we performed a systematic bioinformatics investigation of quantifiable proteins, encompassing GO annotation, functional classification, functional enrichment and cluster analysis based on functional enrichment.

3.3. GO and KEGG pathway analysis

3.3.1. Functional classification of proteins according to GO annotation

GO analysis revealed that the DEPs were predominantly located in the extracellular space (Fig. 2, A), and were significantly associated with various biological processes, including single-organism process, biological regulation, cellular process, response to stimulus, localization, and metabolic process (Fig. 2, B). To further clarify the molecular functions of the proteins, we carried out a secondary annotation classification through GO and found that these proteins mainly play roles in binding and the regulation of molecular function, as shown in Fig. 2, C. Additionally, we discovered that a significant portion of these proteins are located in the extracellular region, organelles, and cells, as illustrated in Fig. 2, D.

3.3.2. Functional enrichment analysis of DEPs

To determine the significant enrichment tendency of DEPs in specific functional types, we conducted an enrichment analysis of GO, KEGG, domain and other functional annotation types based on the annotations of all identified proteins in 3.3.1 and the screening results of DEPs. The GO enrichment analysis revealed that the identified proteins were significantly enriched in various biological processes, including secretion, platelet degranulation, inflammatory response, regulated exocytosis and other related terms (Supplement Table 4). Additionally, KEGG pathway analysis was conducted to elucidate the functional roles of these modules (Supplement Table 5). The results revealed that the upregulated DEPs were involved in the transcriptional misregulation in cancer pathway (hsa05202), while the downregulated DEPs were enriched in the staphylococcus aureus infection pathway (hsa05150).

Enrichment analysis of protein domains revealed that most DEPs were enriched in VIT domain, inter-alpha-trypsin inhibitor heavy chain C-terminal domain, von Willebrand factor type A domain and serpin domain (Supplement Table 6).

3.3.3. Clustering analysis

For the purpose of exploring functional correlations among DEPs in APS, we categorized them into four clusters based on their APS/NC ratios (differential expression multiple): Q1 ($0 <$ Ratio $\leq 1/1.5$), Q2 ($1/1.5 <$ Ratio $\leq 1/1.2$), Q3 ($1.2 <$ Ratio ≤ 1.5), and Q4 (Ratio >1.5) (Supplement Figure 1). Subsequently, 20 DEPs identified in Q4 underwent further analysis through the STRING database, leading to the development of a protein-protein interaction (PPI) network (https://cn.string-db.org/cgi/network?taskId=bFygREt3vOcc&sessionId=beOt6rBbZV7J&allnodes=1) (Supplement Table 7). Several upregulated DEPs were identified, including serpin peptidase inhibitor clade A member 1 and 2, clade C member 1 (SERPINC1/A1/A7), apolipoprotein A1, A2, H (APOA1/2 and H), orosomucoid 1 and 2 (ORM1/2), transferrin (TF) and hemopexin (HPX). These DEPs form a tightly interconnected network with the exception of APOH. Additionally, enrichment and cluster analyses were conducted according to GO classification, KEGG pathway, and protein domain to elucidate differences in biological terms enriched by each cluster. The KEGG pathway analysis revealed that the proteins in Q4 were significantly enriched in the peroxisome proliferator-activated receptors (PPAR) signaling pathway, staphylococcus aureus infection, and African trypanosomiasis terms (Fig. 3, A). Additionally, protein domain analysis revealed that the DEPs in cluster Q2 were significantly associated with fibrinogen, serpin domain, macroglobulin, netrin domain, and C−type lectin fold (Fig. 3, B). This further suggests that DEPs in Q2 play a crucial role in enzyme inhibitor activity, particularly serine-type endopeptidase inhibitor activity. Conversely, the proteins in Q4 were primarily enriched in regulating response to glycerolipid biosynthetic process, vitamin transport and negative regulation (Fig. 3, C).

Regarding molecular function, the proteins in Q4 exhibited significant enrichment in various molecular functions, including serine-type endopeptidase inhibitor activity, peptidase inhibitor activity, endopeptidase regulator activity, enzyme regulator activity, enzyme inhibitor activity and molecular function regulation (Fig. 3, D). Regarding cellular components (Fig. 3, E), numerous DEPs in Q4 were found to be enriched in various types of vesicles, including endocytic, intracellular, and cytoplasmic vesicles. This suggests that the transportation of proteins via exosomes may play a significant role in the mechanism underlying APS pathology.

4. Discussion

Previous research has indicated that plasm exosomes play a crucial role in systemic signaling events in APS [22], and their component alterations can increase the risk of pregnancy complications. In OAPS patients' plasm, exosomes perform various biological functions through their numerous enclosed cargoes. Exosomes are secreted by cells and contain a diverse array of molecules that collaborate to orchestrate precise cellular responses to environmental changes or stress signals. Investigation into the protein composition of plasm exosomes from early pregnant women with and without APS will facilitate identification of key biomarkers, associated signaling pathways, and biological processes. To comprehensively analyze the protein signatures of participants' plasm exosomes and gain a systematic understanding of APS development during pregnancy, we conducted an extensive proteomics analysis in this study. By conducting proteomics analysis of the plasma exosomes from APS patients and healthy pregnant women, we identified 73 DEPs, such as SERPINC1/A1/A7, APOA1/2, ORM1/2, and APOH. These DEPs are primarily associated with anticoagulation, lipid metabolism, inflammatory responses, and complement activation. Enrichment analysis suggests that they are linked to the biosynthesis of glycerolipids, vitamin transport, and the negative regulation of very low-density lipoprotein particle remodeling. Pathway analysis indicated potential underlying mechanisms through which DEPs may influence APS pathology, such as the PPAR and *Staphylococcus aureus* infection signaling pathways, although further experimental validation is required.

Notably, we observed an upregulation of SERPIN family members, including SERPINA1, SERPINA7, and SERPINC1 in comparison to the NC group. This finding underscores the potential involvement of exosomes derived from APS individuals in coagulation. SERINC1 is also known as serpin peptidase inhibitor clade C (antithrombin) member 1. Numerous studies have confirmed that variants of SERPINC1 play crucial roles in the development of diseases related to the coagulation system, including antithrombin deficiency and severe thrombophilia [15,23]. Additionally, some homozygous variant of *serpinc1* are strictly associated with adverse pregnancy outcomes [24], suggesting that plasm exosomes participate in regulating the coagulation system during the development of OAPS. In addition, SERPINA1 and plasminogen have been identified as novel biomarkers in OAPS patients [3]. Furthermore, deleterious variants of *serpina1* can compromise the structures essential for maintaining pregnancy against proteases and inflammatory activation, leading to spontaneous preterm delivery [25]. However, SERPINA7, another member of the SERPIN family, is primarily involved in the synthesis of thyroxine-binding globulin and has not yet been reported to be associated with APS or pregnancy.

Numerous studies have confirmed the crucial roles of lipid metabolism in early embryo development, implantation, and uterine receptivity [26]. Meanwhile, dysregulated lipid metabolism is associated with the development and poorer prognosis of APS and thrombosis [27,28]. Our proteomics research has revealed a tight association between plasm exosomes from APS pregnant individuals and lipid metabolism. GO analysis revealed that the plasm exosomes of individuals with APS in early pregnancy exhibited upregulation of proteins significantly involved in regulating phospholipid and glycerolipid biosynthetic processes, as well as negatively regulating very-low-density lipoprotein particle remodeling. Additionally, the APS group exhibited significant upregulation of APOA1/2 and APOH. APOA1/2 is a lipoprotein transporter whose antibodies have been described in APS [29]. Furthermore, gestational women's plasm regularly comprises high levels of lipoprotein [30]. APOH, also known as β2GPI, is a crucial antigen in the pathogenesis of APS, and the antigen-antibody complex of antiphospholipid antibodies and APOH increases the affinity for negatively charged phospholipids on the cell surface, thereby activating endothelial cells, mononuclear cells and platelets [11,31]. Furthermore, it plays a pivotal role in various physiological pathways such as lipoprotein metabolism, coagulation cascade, hemostasis regulation and the production of antiphospholipid autoantibodies. Previous research has demonstrated that APOH may induce endothelial dysfunction and affect angiogenesis by inhibiting *P*-Erk1/2 signaling [11].

ORM1/2, an acute-phase reactant involved in immunosuppression, is upregulated during acute inflammation [32]. TF acts as a granulocyte/pollen-binding protein (GPBP) and plays a physiological role in removing allergens from the plasm [33]. Our study found that ORM1/2 and TF were upregulated in plasm exosomes of APS patients, suggesting that dysfunction of inflammatory reaction mediated by exosomal cargos may be involved in the development of APS during early pregnancy.

Moreover, KEGG analysis revealed that the DEPs were significantly enriched in the PPAR signaling pathway, which consists of three subtypes [34]. Previous studies have indicated that PPARγ is expressed in the placenta and exerts a significant impact on both its development and function [35]. Moreover, PPAR promotes a gradual decline in placental lipid concentration throughout pregnancy and plays a crucial role in the etiology of various adverse outcomes during the perinatal period due to detrimental exposure in the first trimester [36,37]. The effectiveness of PPAR as a biomarker for OAPS remains uncertain, however, this study presents a promising perspective.

Importantly, a set of down-regulated DEPs that participated in the complement and coagulation cascades were found to be enriched in the *Staphylococcus aureus* infection signaling pathway. These DEPs including complement component 4 (C4), complement C1q subcomponent subunit A (C1QA), which involved in activation of the classical complement pathway, C3, which was composed of the alternative pathway, complement factor H (HF) and mannan-binding lectin serine protease 1 (MASP1/2). In contrast, C2 exhibited upregulation. Additionally, previous study indicated that decreased plasm levels of C4 and C3 have been reported in approximately one third of patients with APS [38]. The complement system plays a double role in pregnancy exerting both protective and damaging effects at placental level, and participants in the regulation of immunological tolerance in pregnancy [39]. In the early stage of pregnancy, the fetus and the placenta might be subject to complement-mediated immune attack with the potential risk of adverse pregnancy outcomes [40,41]. These indicated that the cargos of plasm exosomes may play a crucial role in the development of APS pathology by influencing complement activation.

With various biological macromolecules, such as proteins, lipids, DNA, RNA and more, exosomes have the potential to serve as biomarkers or therapeutic targets for female reproductive diseases including polycystic ovarian syndrome (PCOS), reproductive cancer and preeclampsia [42]. However, current clinical therapeutic methods targeting OAPS have yet to achieve perfection. Despite

receiving treatment, pregnancy complications may still arise and second-line interventions may be necessary. Our analysis of plasm exosomes from OAPS patients serves as a promising starting point for future research into innovative approaches for early detection and prompt intervention. The results of our proteomics investigation on plasm exosomes will shed light on the underlying mechanisms of OAPS onset and facilitate the identification of relevant biomarkers in plasm exosomes from OAPS patients.

However, this study also has several limitations. Firstly, although our research initially discovered some exosomal proteins meaningful for OAPS, the study did not validate the identified biomarkers in an independent cohort, which is crucial for determining the reliability and applicability of the research findings. Validation in larger, independent cohorts is required before these proteins can be considered reliable biomarkers for clinical use. Secondly, the sample size of our study is relatively small, with only 6 participants per group. This limits the scope of the applicable population and may affect the generalizability of the results. Future studies need larger cohorts to validate these preliminary findings. Thirdly, this study is a cross-sectional design, capturing only a single time point in early pregnancy and lacks longitudinal data to analyze how exosomal protein expression changes throughout pregnancy and how these changes relate to APS progression or pregnancy outcomes. Fourth, the study population may lack diversity in ethnicity, geographical location, and other potential confounding factors such as socioeconomic status or baseline health conditions. Future studies need to include a more diverse participant pool to ensure the research findings are applicable to different populations. Fifth, although the study controlled for some variables (age, gestational age, and APS treatment regimen), there may be other confounding factors not considered, such as participants' lifestyle factors, nutritional status, or Body Mass Index (BMI). Future research needs to control for these potential confounders. Sixth, unlike other autoimmune diseases or pregnancy-related diseases, the specificity of the identified biomarkers for APS has not been thoroughly investigated. Further research is needed to determine whether these biomarkers are unique to APS or if they may also be elevated in other autoimmune or pregnancy-related diseases. Finally, the study relies on specific proteomics and bioinformatic techniques, which, although advanced, have their limitations in terms of sensitivity, specificity, and potential bias.

Our study also has notable strengths. Firstly, the use of proteomics analysis of serum exosomes as biomarkers for early-stage APS is a novel and meaningful approach, providing new insights for the early diagnosis of the disease. Secondly, the application of advanced proteomics and bioinformatics analysis is a key advantage for the detailed characterization of the exosomal proteome. The depth and breadth of these analyses offer a rich dataset for identifying potential biomarkers. Utilizing LC-MS techniques identified a large number of proteins, offering a more comprehensive understanding of the molecular basis of the disease. Thirdly, the findings of this study lay a solid foundation for future research on the pathophysiology of APS, as well as the development of diagnostic and therapeutic strategies. The biomarkers identified in this study have the potential to improve the early diagnosis of APS during pregnancy and guide treatment strategies. Lastly, this research not only contributes to the understanding of APS during pregnancy but also lays the groundwork for future studies. Early detection and timely intervention could improve the pregnancy outcomes of women with APS. Future studies might independently validate the identified differential proteins in an expanded cohort, constructing models of these biomarkers for the early diagnosis of OAPS, thereby improving early detection or guiding therapeutic interventions.

5. Conclusion

In conclusion, we conducted a comprehensive proteomics investigation of plasm exosomes derived from both OAPS patients and healthy pregnant women in the first trimester. Our study has identified potentially significant biomarkers, such as SERPINC1/A1/A7, APOA1/2, ORM1/2, and APOH. These plasma exosomal proteins may play roles in various processes, including anticoagulation, lipid metabolism, inflammatory responses, complement activation, and related signaling pathways. However, further research and validation are necessary. The results of our study may offer references for exploring early diagnostic biomarkers for OAPS. Additional research is needed to determine the mechanisms by which exosomal proteins influence OAPS, as well as to investigate potential biomarkers for the early detection and treatment of this condition.

Data availability statement

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Ethics approval

This study was approved by the Ethics Committee of Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine (Ethics Approval Number: KS22212). All participants in the study signed an informed consent form, and we adhered to ethical guidelines throughout the research process.

CRediT authorship contribution statement

Yeli Sun: Writing – original draft. **Zheng Wang:** Data curation. **Junyuan Li:** Conceptualization. **Tongshuai Wang:** Conceptualization. **Yuan Tan:** Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29224.

References

[1] A. Capozzi, V. Manganelli, G. Riitano, S. Recalchi, S. Truglia, C. Alessandri, A. Longo, T. Garofalo, R. Misasi, G. Valesini, F. Conti, M. Sorice, Tissue factor over-expression in platelets of patients with anti-phospholipid syndrome: induction role of anti-beta2-GPI antibodies, Clin. Exp. Immunol. 196 (2019) 59–66, 10/gtmtvr.

[2] I.J. Walter, M.J. Klein Haneveld, A.T. Lely, K.W.M. Bloemenkamp, M. Limper, J. Kooiman, Pregnancy outcome predictors in antiphospholipid syndrome: a systematic review and meta-analysis, Autoimmun. Rev. 20 (2021) 102901, 10/gtmvd2.

[3] M. Wang, P. Zhang, S. Yu, G. Zhou, J. Lv, D. Nallapothula, C. Guo, Q. Wang, R.R. Singh, Heparin and aspirin combination therapy restores T-cell phenotype in pregnant patients with antiphospholipid syndrome-related recurrent pregnancy loss, Clin. Immunol. 208 (2019) 108259, 10/gtmtwb.

[4] D. Garcia, D. Erkan, Diagnosis and management of the antiphospholipid syndrome, N. Engl. J. Med. 378 (2018) 2010–2021, 10/gf6g9z.

[5] A. Antovic, M. Sennstrom, K. Bremme, E. Svenungsson, Obstetric antiphospholipid syndrome, Lupus Sci. Med. 5 (2018) e000197, 10/gfh78c.

[6] E.N. Hamulyak, L.J.J. Scheres, M. Goddijn, S. Middeldorp, Antithrombotic therapy to prevent recurrent pregnancy loss in antiphospholipid syndrome-What is the evidence? J. Thromb. Haemostasis 19 (2021) 1174–1185, 10/grxb5s.

[7] L.A. Coletto, M. Gerosa, M. Valentini, R. Cimaz, R. Caporali, P.L. Meroni, C.B. Chighizola, Myocardial involvement in anti-phospholipid syndrome: beyond acute myocardial infarction, Autoimmun. Rev. 21 (2022) 102990, 10/grbw38.

[8] R.D. Pablo, P.M. Cacho, M. Lopez-Hoyos, V. Calvo-Rio, L. Riancho-Zarrabeitia, V.M. Martinez-Taboada, Risk factors for the development of the disease in antiphospholipid antibodies carriers: a long-term follow-up study, Clin. Rev. Allergy Immunol. 62 (2022) 354–362, 10/gtmtv5.

[9] M. Gerde, E. Ibarra, R. Mac Kenzie, C. Fernandez Suarez, C. Heer, R. Alvarez, M. Iglesias, J. Balparda, E. Beruti, F. Rubinstein, The impact of hydroxychloroquine on obstetric outcomes in refractory obstetric antiphospholipid syndrome, Thromb. Res. 206 (2021) 104–110, 10/gtmtvv.

[10] M.G. Tektonidou, L. Andreoli, M. Limper, Z. Amoura, R. Cervera, N. Costedoat-Chalumeau, M.J. Cuadrado, T. Dorner, R. Ferrer-Oliveras, K. Hambly, M. A. Khamashta, J. King, F. Marchiori, P.L. Meroni, M. Mosca, V. Pengo, L. Raio, G. Ruiz-Irastorza, Y. Shoenfeld, L. Stojanovich, E. Svenungsson, D. Wahl, A. Tincani, M.M. Ward, EULAR recommendations for the management of antiphospholipid syndrome in adults, Ann. Rheum. Dis. 78 (2019) 1296–1304, 10/gf878f.

[11] Y. Tan, Y. Bian, Y. Song, Q. Zhang, X. Wan, Exosome-contained APOH associated with antiphospholipid syndrome, Front. Immunol. 12 (2021) 604222, 10/gtmtv9.

[12] M. Mazzariol, G. Camussi, M.F. Brizzi, Extracellular vesicles tune the immune system in renal disease: a focus on systemic lupus erythematosus, antiphospholipid syndrome, thrombotic microangiopathy and ANCA-vasculitis, Int. J. Mol. Sci. 22 (2021), 10/gtmtv4.

[13] S. Chaturvedi, R. Alluri, K.R. McCrae, Extracellular vesicles in the antiphospholipid syndrome, Semin. Thromb. Hemost. 44 (2018) 493–504, 10/gdt3zf.

[14] B. Chen, L. Song, J. Yang, W.Y. Zhou, Y.Y. Cheng, Y.J. Lai, Proteomics of serum exosomes identified fibulin-1 as a novel biomarker for mild cognitive impairment, Neural Regen. Res. 18 (2023) 587–593, 10/gtmtvs.

[15] M.E. de la Morena-Barrio, P. Suchon, E.M. Jacobsen, N. Iversen, A. Minano, B. de la Morena-Barrio, C. Bravo-Perez, J. Padilla, R. Cifuentes, S. Asenjo, J. F. Deleuze, D.A. Tregouet, M.L. Lozano, V. Vicente, P.M. Sandset, P.E. Morange, J. Corral, Two SERPINC1 variants affecting N-glycosylation of Asn224 cause severe thrombophilia not detected by functional assays, Blood 140 (2022) 140–151, 10/gqjmh8.

[16] L. Zhang, D. Yu, Exosomes in cancer development, metastasis, and immunity, Biochim. Biophys. Acta Rev. Canc 1871 (2019) 455–468, 10/gg376v.

[17] Evaluation and Treatment of Recurrent Pregnancy Loss: a Committee opinion., United States, 2012, https://doi.org/10.1016/j.fertnstert.2012.06.048.

[18] C. Hu, S. Li, Z. Xie, H. You, H. Jiang, Y. Shi, W. Qi, J. Zhao, Q. Wang, X. Tian, M. Li, Y. Zhao, X. Zeng, Comparison of different test systems for the detection of antiphospholipid antibodies in a Chinese cohort, Front. Immunol. 12 (2021) 648881, 10/gtmvq7.

[19] M. Harel, R. Ortenberg, S.K. Varanasi, K.C. Mangalhara, M. Mardamshina, E. Markovits, E.N. Baruch, V. Tripple, M. Arama-Chayoth, E. Greenberg, A. Shenoy, R. Ayasun, N. Knafo, S. Xu, L. Anafi, G. Yanovich-Arad, G.D. Barnabas, S. Ashkenazi, M.J. Besser, J. Schachter, M. Bosenberg, G.S. Shadel, I. Barshack, S. M. Kaech, G. Markel, T. Geiger, Proteomics of melanoma response to immunotherapy reveals mitochondrial dependence, Cell 179 (2019) 236–250.e18, 10/gf97x3.

[20] M. Kanehisa, S. Goto, KEGG: kyoto encyclopedia of genes and genomes, Nucleic Acids Res. 28 (2000) 27–30, 10/b9st54.

[21] D. Szklarczyk, R. Kirsch, M. Koutrouli, K. Nastou, F. Mehryary, R. Hachilif, A.L. Gable, T. Fang, N.T. Doncheva, S. Pyysalo, P. Bork, L.J. Jensen, C. von Mering, The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest, Nucleic Acids Res. 51 (2023) D638–D646, 10/gs7sn3.

[22] S.M. Gysler, M.J. Mulla, M. Guerra, J.J. Brosens, J.E. Salmon, L.W. Chamley, V.M. Abrahams, Antiphospholipid antibody-induced miR-146a-3p drives trophoblast interleukin-8 secretion through activation of Toll-like receptor 8, Mol. Hum. Reprod. 22 (2016) 465–474, 10/gtmtvz.

[23] D. Provaznikova, M. Matyskova, I. Capova, D. Grancarova, E. Drbohlavova, M. Slechtova, I. Hrachovinova, Seventeen novel SERPINC1 variants causing hereditary antithrombin deficiency in a Czech population, Thromb. Res. 189 (2020) 39–41, 10/gtmtv7.

[24] M. Kovac, G. Mitic, Z. Mikovic, V. Mandic, P. Miljic, M. Mitrovic, B. Tomic, Z. Bereczky, The influence of specific mutations in the AT gene (SERPINC1) on the type of pregnancy related complications, Thromb. Res. 173 (2019) 12–19, 10/gqxx4h.

[25] H. Tiensuu, A.M. Haapalainen, P. Tissarinen, A. Pasanen, T.A. Maatta, J.M. Huusko, S. Ohlmeier, U. Bergmann, M. Ojaniemi, L.J. Muglia, M. Hallman, M. Ramet, Human placental proteomics and exon variant studies link AAT/SERPINA1 with spontaneous preterm birth, BMC Med. 20 (2022) 141, 10/gqxqpc.

[26] Q. Ye, X. Zeng, S. Cai, S. Qiao, X. Zeng, Mechanisms of lipid metabolism in uterine receptivity and embryo development, Trends Endocrinol. Metabol. 32 (2021) 1015–1030, 10/gqwk4c.

[27] F. Mu, Y. Wang, H. Wu, Q. You, D. Zhang, The myocardial infarction-associated transcript 2 inhibits lipid accumulation and promotes cholesterol efflux in oxidized low-density lipoprotein-induced THP-1-derived macrophages via inhibiting mitogen-activated protein kinase signaling and activating the nuclear factor erythroid-related factor 2 signaling pathway, Bioengineered 13 (2022) 407–417, 10/gqgwrg.

[28] T.C.R. Rodrigues, C. de Oliveira Vaz, E.C.M. Miranda, M. Pereira, S. da Silva Saraiva, J.M. Annichino-Bizzacchi, B. de Moraes Mazetto, F.A. Orsi, Efficacy of a hypolipid diet in patients with primary antiphospholipid syndrome with dyslipidemia: a prospective study, J. Thromb. Thrombolysis 53 (2022) 390–398, 10/gtmtv8.
[29] N. Vuilleumier, G. Reber, R. James, D. Burger, P. de Moerloose, J.M. Dayer, P. Roux-Lombard, Presence of autoantibodies to apolipoprotein A-1 in patients with acute coronary syndrome further links autoimmunity to cardiovascular disease, J. Autoimmun. 23 (2004) 353–360, 10/cznhhz.
[30] L. Hillman, G. Schonfeld, J.P. Miller, G. Wulff, Apolipoproteins in human pregnancy, Metabolism 24 (1975) 943–952, 10/bxtzm6.
[31] H. Mehdi, C.E. Aston, D.K. Sanghera, R.F. Hamman, M.I. Kamboh, Genetic variation in the apolipoprotein H (beta2-glycoprotein I) gene affects plasma apolipoprotein H concentrations, Hum. Genet. 105 (1999) 63–71, 10/d57chx.
[32] D.L. Siow, B.W. Wattenberg, Mammalian ORMDL proteins mediate the feedback response in ceramide biosynthesis, J. Biol. Chem. 287 (2012) 40198–40204, 10/f4f6m2.
[33] B. Østerud, N. Latysheva, C. Schoergenhofer, B. Jilma, J.-B. Hansen, O. Snir, A rapid, sensitive, and specific assay to measure TF activity based on chromogenic determination of thrombin generation, J. Thromb. Haemostasis 20 (2022) 866–876, 10/gtmvt2.
[34] G. Yu, J. Wang, Y. Liu, T. Luo, X. Meng, R. Zhang, B. Huang, Y. Sun, J. Zhang, Metabolic perturbations in pregnant rats exposed to low-dose perfluorooctanesulfonic acid: an integrated multi-omics analysis, Environ. Int. 173 (2023) 107851, 10/gtmtwd.
[35] C. Giaginis, E. Spanopoulou, S. Theocharis, PPAR-gamma signaling pathway in placental development and function: a potential therapeutic target in the treatment of gestational diseases, Expert Opin. Ther. Targets 12 (2008) 1049–1063, 10/cdbdsr.
[36] L. Zhao, X. Zheng, J. Liu, R. Zheng, R. Yang, Y. Wang, L. Sun, PPAR signaling pathway in the first trimester placenta from in vitro fertilization and embryo transfer, Biomed. Pharmacother. 118 (2019) 109251, 10/gtmtwg.
[37] F. Liu, W. Zhu, H. Shoaito, A. Chissey, S.A. Degrelle, T. Fournier, Mining of combined human placental gene expression data across pregnancy, applied to PPAR signaling pathway, Placenta 99 (2020) 157–165, 10/gtmtv2.
[38] C.B. Chighizola, P.A. Lonati, L. Trespidi, P.L. Meroni, F. Tedesco, The complement system in the pathophysiology of pregnancy and in systemic autoimmune rheumatic diseases during pregnancy, Front. Immunol. 11 (2020) 2084, 10/gn7f3j.
[39] L. Teirila, J. Heikkinen-Eloranta, J. Kotimaa, S. Meri, A.I. Lokki, Regulation of the complement system and immunological tolerance in pregnancy, Semin. Immunol. 45 (2019) 101337, 10/gpg7cq.
[40] G. Girardi, Complement activation, a threat to pregnancy, Semin. Immunopathol. 40 (2018) 103–111, 10/gtmtvw.
[41] G. Girardi, J.J. Lingo, S.D. Fleming, J.F. Regal, Essential role of complement in pregnancy: from implantation to parturition and beyond, Front. Immunol. 11 (2020) 1681, 10/gtmtvx.
[42] S. Esfandyari, H. Elkafas, R.M. Chugh, H.S. Park, A. Navarro, A. Al-Hendy, Exosomes as biomarkers for female reproductive diseases diagnosis and therapy, Int. J. Mol. Sci. 22 (2021), 10/gkzk2x.